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Grinding and cooking dry-fractionated corn germ to optimize aqueous enzymatic oil extraction☆

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ABSTRACT

The many recent dry-grind plants that convert corn to ethanol are potential sources of substantial amounts of corn oil. This report describes an aqueous enzymatic extraction method to separate oil from dry-fractionated corn germ. The method is an extension of a method previously developed for wet-mill germ. Oil dispersed in lipid bodies throughout the germ was converted to oil droplets suspended in an aqueous solution and then to drops of oil large enough to be separated from the solution as a continuous, buoyant phase (free oil). A microwave oven was used to cook the germ to its highest temperature, just short of burning. Thereafter the germ was extracted using the method developed for wet-mill germ: mix the heated germ with water and cook it under pressure, followed by colloid milling and enzymatic digestion of the milled germ particles overnight. A foam fraction was removed from the digested dispersion by bubbling nitrogen through a short column connected to a mixing tank. The foam fraction was then centrifuged to separate free oil. An estimate of aqueous enzymatic extraction plant costs to extract 24 million kg of dry-fractionated germ per year [40 million gal/year ethanol], showed that income from the unrefined oil streams and a stream sending the rest of the germ to the fermentation process was roughly equal to the estimated operating cost, with an investment of $2.6 million. Recycle of the enzyme may reduce the estimated enzyme cost of $750,000/year.

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1. Introduction

Corn dry-grind ethanol plants are economically stressed when the corn price rises faster than the ethanol price. At current corn/ethanol price ratios, it may be more profitable for dry-grind plants that produce a single coproduct, distillers dried grains and solubles (DDGS), to add fractionation equipment and produce germ and bran coproducts. Crude corn oil currently costs are estimated to range from 0.39 to 0.42US$/lb [0.86–0.92 US$/kg] (Ash et al., 2010). If 1.0 kg of oil is recovered from each bushel of corn converted by every US dry-grind plant, then 480 million gallons [1820 million L] of crude corn oil could be recovered. To put this number in context, 324 million gallons [1227 million L] of corn oil are estimated to be extracted in the US in 2009/2010 (ERS, 2009). Processes have been developed to separate corn germ with increasingly higher purity: Quick germ (Singh and Eckhoff, 1996), enzymatically milled germ (Johnston et al., 2005) and MOR-Frac, a dry-milling followed by wet-mill refining (Reidy, 2009) report germs with 30, 39 and 42% oil, respectively. These processes are less expensive to retro-fit an existing dry-grind plant than to invest in the germ separation equipment (including steeping) required for full scale wet-milling. Traditionally oil recovery from the germ, including germ from dry-mill plants whose primary product is the grits used for food, has been limited to large and expensive hexane extraction plants at a scale well above that of most dry-grind ethanol plants. Thus the incentive to retro-fit a dry-grind plant with germ separation equipment would be increased if the feasibility of small scale germ extraction was shown. Economical germ extraction, after separation, would enable the plant to produce an oil coproduct independent of the hexane extraction plants, which are now operating near capacity.

Our aqueous enzymatic extraction corn germ project began using wet-milled germ since it has the highest levels of oil (40–50%) and is easiest to extract. Good oil yields were obtained from wet-mill germ using an aqueous enzymatic process (Moreau et al., 2004), and the oil extraction cost was estimated to be comparable to the cost of expelling (Dickey et al., 2009). However preliminary tests showed that aqueous enzymatic extraction, as developed for wet-mill germ is ineffective with dry-fractionated germ (which contains 15–20% oil); no oil was extracted from dry-fractionated germ using aqueous enzymatic extraction optimized for wet-mill germ, which included a 20 min cook of a 10% dispersion of the germ in water at 2 atm (120 °C), followed by grinding of the germ particles in the

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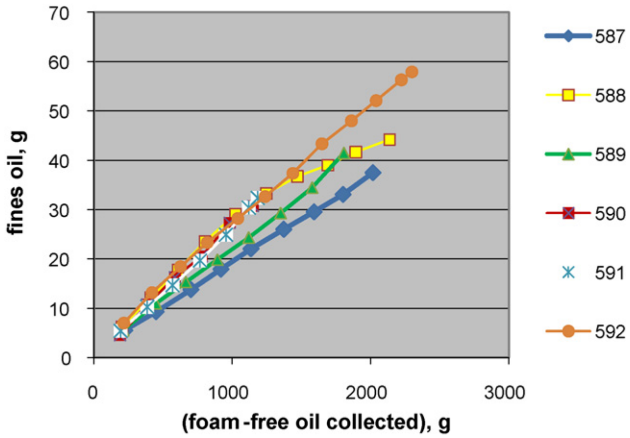

Fig. 1. Free oil fraction of total oil in foam. Run conditions are described in Tables 1 and 2.

dispersion in a colloid mill for 3 min and overnight digestion of the dispersion with Accellerase™ 1000 enzyme complex. An approach that has been recently reported improves aqueous extraction yields for dry-fractionated germ by using enzyme(s) selected to weaken or degrade oil bodies present in the germ. The oil bodies may be degraded in wet-mill germ during steeping and or drying of the kernels of the germ (Moreau et al., 2009).

A different approach, described here, is to cook the germ prior to aqueous enzymatic extraction of the germ. Since dry-fractionated germ is a dry material use of a dry heating method such as a microwave oven is simple and increases germ temperatures up to 180 °C without burning, at a cost unattainable in an aqueous solution, without cost-prohibitive pressure (10 atm). We hypothesized that heating experienced by wet-mill germ during its drying, improves its oil extractability which may already have been improved by steeping. The experiments described here are extensions of the method based on heating wet-mill germ to improve aqueous enzymatic extraction oil yield using a new commercial cellulase Accellerase™ 1000 enzyme complex.

2. Materials and methods

2.1. Materials

Batches of dry-fractionated germ were obtained from commercial mills.

Bunge USA (Danville, IL) [2.9 ± 0.3% moisture, 15.8 ± 0.4% lipid], and Cereal Process Technologies, LLC (Overland Park, KS) [2.7 ± 1.2% moisture, 17.2 ± 1.7% lipid] and stored in polyethylene bags, inside sealed drums, at 4 °C until needed. Accellerase™ 1000 enzyme complex (Genencor, a Danisco Division) was kept refrigerated until used.

2.1.1. AEE extraction equipment

A household microwave oven with a rotating stand (Panasonic model S954WF, 1250 W, 2450 MHz, Secaucus, NJ) was used to cook the dry-milled germ prior to preparation of the dispersion. A 25.4 cm spring-wound turntable (Micro Go Round, NordicWare, Minneapolis, MN) was used on some later runs, mounted on the periphery of the built-in rotating stand to vary germ exposure to (possibly) different irradiation intensities during its cycloidal path in oven. A Hermle Z383K centrifuge with swing-out rotor (Labnet International, Woodbridge, NJ) was used to separate the contents of dispersion samples into density-based fractions.

2.2. Experimental

In a standard run, 400 g batches of dry-milled germ were spread evenly to a depth of 2–3 cm, in a 2.6-l rectangular Pyrex dish and heated in a microwave oven. After the microwave heating, the aqueous enzymatic extraction process developed for wet-mill germ was used (Dickey et al., 2009); this consisted of mixing the heated germ with 4 kg of water and cooking, at 2 atm, in a pressure cooker for 20 min, grinding in a colloid mill for 3 min, cooling for 30 min, and adding about 160 mL water (restoring water lost during pressure cook). Two 400 g batches of germ dispersion were mixed in a 20 L stirred polypropylene pail with 10 ml of enzyme solution at 50 °C for 24 h. The dispersion was then pumped through the bottom section of the bubble column described previously (Dickey et al., 2009) and the foam overflow collected in approximately 200 g increments until foaming ceased (Fig. 1). The foam was more stable than might be expected and was stabilized by compounds in the dispersion released from the germ, tentatively identified as lipid-transfer proteins isolated from diverse plants, including corn (Dickey et al., 2009). The collected foam samples were separately centrifuged at 4211 × *g* for 60 min to remove non-buoyant solid particles, then the centrate was frozen and a free oil layer (paste) scraped from the hard ice, with a spatula. The oil paste was melted, lyophilized (Virtis, Gardiner, NY), weighed and the free oil content measured by hexane extraction (Moreau et al., 2003). The centrate and solid particle fractions for each sample, were melted, re-centrifuged, then weighed, dried and analyzed for oil and protein. The lipid mass in the liquid left after free oil removal was termed fines oil.

A run was made in which germ was colloid milled and drained through 2 shaker sieves (#14/#100) for 1 h. The wet particles were spread out on flat surfaces to dry overnight and the two batches of drained liquid were evaporated over hot plates. The particles lost considerable weight but had not completely dried and never reached the high temperature typically observed with whole germ or dry particles during microwave heating at 50% power (40 min) with intermittent stops to hand mix the milled germ.

3. Results and discussion

3.1. Background studies with Accellerase™ 1000

In previous experiments, oil was extracted from 800 g batches of wet-milled corn germ and recoveries of 80% of the hexane extractable-oil were obtained, mostly as free oil. Enzyme solution-to-germ mass ratios of 1:10, 1:20, 1:40 and 1:80 and 1:160 were evaluated and the 1:80 mass ratio was found to give the best oil yields. The optimal pH of the wet-mill germ dispersion was about 4.0 prior to separation of the foam fraction (Dickey et al., 2009). The procedure developed to extract oil from wet-mill germ with aqueous enzymes did not work with dry-fractionated germ; when the enzyme solution-to-germ mass ratio was raised to 3:80 the yield was 9%. Dry-fractionated germ/water dispersions have a pH of 6 before adding enzyme and decrease to around 4.6 after adding enzyme (1:80 mass ratio). When the germ dispersion pH was lowered to 4.5 with sulfuric acid, before adding enzyme, there was no improvement in free oil yield. Heating to remove oil from corn germ is required by all procedures, including expelling (where the heat is supplied by compression) and hexane extraction. Although the aqueous enzymatic extraction procedure that was developed for wet-mill germ includes an initial 120 °C cook, this temperature is not high enough to release oil from dry-fractionated germ, with practical amounts of enzyme.

Table 1
Foam, free oil and fines oil yield produced by aqueous enzymatic extraction of 800 g of microwave-cooked dry-fractionated germ.

Run	Heating period (min)	Power setting (%)	Foam collected (g)	Free oil (g)		Fines oil (g)	
				Foam	Non-foam	Foam	Non-foam
574	8	100	1106.9	28.7	na	38.4	na
575	8[a]	100	963.4	16.7	na	29.9	na
577	28[b]	100	0	0	14	0	25.8
578	10[c]	100	1205.6	29.6	1.1	44.5	24.3
579	10[d]	100	1322.8	26.3	4.7	47.1	20.5
581	16[e]	50	1339.8	40.0	2.9	43.3	8.6
583	7	100	1352.6	29.5	3.4	48.4	4.9
	20	10					
584	7	100	1380	26.6	4.2	49.7	8.9
	20	30					
585	10[f]	30	1150	28.7	9.3	37.3	8.5

A mass ratio of 10:1 water to germ was used to cook (2 atm) the germ after microwave heating and before colloid milling (3 min) the cooked germ. A mass ratio of 1:80 enzyme complex to germ was used to digest the germ dispersion over night prior to foam collection.

[a] Germ soaked in 1.5 L of water (initially 70 °C, but cooled to ambient overnight) then drained (#100 sieve, 1 h) before microwave heating.

[b] Germ was heated for 14 min at full power then removed from oven, mixed by hand, then returned to oven for 14 min more heat at full power.

[c] Germ was cooled for 1 h after 5 min at full power in oven and then heated for another 5 min at full power. Dispersion pH was reduced to 4 with sulfuric acid prior to pressure cooking.

[d] Germ was cooled for 1 h after heating 5 min at full power and then heated for another 5 min at full power. Dispersion pH was reduced to 4.1 over 4 h after colloid milling and dispersion cooling in the mixing tank.

[e] Germ was heated 8 min at 50% power, removed from oven, mixed by hand, heated 8 min at 50% power.

[f] Germ was cooled for 30 min after heating (5 min) at full power and heated for 5 min at full power. 250 g foam produced, the non-foam was returned to the mixing tank, 20 ml of enzyme added and the dispersion foamed the next morning.

3.2. Studies with dry-fractionated germ, variation of microwave heating time and power

For the first experiments with dry-fractionated germ, the germ was heated in a microwave oven and then dispersed in water and colloid milled. Preliminary tests showed that microwave heating for more than 8 min at full power would burn some particles, despite rotation of the turntable. The temperature of the germ at the end of the cook, measured with a hand-held thermocouple, was around 150 °C. Several changes in heating schedule followed by the wet-mill germ extraction procedure were tried in order to determine the schedule that gave the highest free oil yield. Table 1 summarizes the results of those tests. The best results were obtained from a 16 min cook at 50% power, with mixing half way through: 8 min of microwave heating at 50% power followed by mixing of the germ with a spatula, temperature measurement (99 °C) and continued heating at 50% power for 8 min. The final temperature of the germ was 144 °C. This (standard) microwave heating was used for subsequent testing of procedures on heated, dry-fractionated germ, before finishing with enzymatic digestion to measure the oil yield and thereby determine the effect of those procedures. Hereafter dry-fractionated germ heated with the standard microwave heating will be called heated dry-fractionated germ.

3.3. Variation of germ treatment after standard microwave heating

Wet-mill germ contains 40–50% oil and dry-milled germ contains 15–20% oil based on hexane extraction; in addition the fraction of the theoretical yield that can be obtained from heated dry-fractionated germ is lower than from wet-mill germ. The initial corn steeping used to produce wet-mill germ may be responsible for its greater extractability. Motivated by this idea another series of runs, using dry-fractionated germ from another source, was carried out to determine the free oil yield changes resulting from different procedures prior to enzyme digestion. Fig. 1 shows that the rate of fines oil recovery was steady for the early foam samples, for all the runs. A run in which the aqueous heating step was elim-

Table 2
Foam, free oil and fines oil yield produced by aqueous enzymatic extraction of 800 g of dry-milled germ cooked for 16 min at 50% power.

Run	Colloid milling (min)	Modifications to standard protocol	Foam collected (g)	Free oil (g)		Fines oil (g)	
				Foam	Non-foam	Foam	Non-foam
588	6[a]	Vacuum dry after 1st colloid	3196.5	67.4	0.0	50.0	2.2
–	3[b]	Milling mill before 2 atm cook	0	–		–	
589x	6[c]	Cook was eliminated	0	–		–	
589	6[d]	Dispersion was cooked at 1 atm	1626.1	44.8	0.9	41.5	8.4
590	30[e]	No cooling before 2 atm cook	1190.7	40.7	12.9	30.7	8.8
591	3[f]	No cooling before 2 atm cook, 2nd turntable added	1238.2	54.3	6.4	32.3	0.8
592	3[f]	Same as 591 except 2.5 L water used to cook germ	2346	48.2	0.4	57.9	2.7

A mass ratio of 10:1 water to germ was used to cook the germ after microwave heating and to colloid mill the cooked germ. A mass ratio of 1:80 enzyme complex to germ was used to digest the germ dispersion over night prior to collection of the foam.

[a] Germ was mixed with 10 water mass and colloid milled 3 min and vacuum-dried before microwave heating (16 min at 50% power), pressure cooking (10% germ in water, 2 atm, 20 min), colloid milled (3 min) and digested with enzyme.

[b] Germ was mixed with 10 water mass and colloid milled 3 min before 2 atm cook. The dispersion foamed in the pressure cooker blowing particles and liquid through relief valve.

[c] Germ was mixed with 10 water mass and colloid milled 6 min then pumped into mixing tank with 10 ml of enzyme and mixed over night. No foam resulted from bubbling. Dispersion pH was 6.7.

[d] Dispersion from 589× was cooked at 1 atm, 20 min, cooled to 50 °C, and 10 ml of enzyme was added.

[e] Germ was transferred rapidly from mw oven to pressure cooker, and after 20 min cook, colloid milled 30 min.

[f] Germ was transferred rapidly from mw oven to pressure cooker, and after 20 min cook, colloid milled 3 min. A 2nd turntable was set on the turntable supplied with the microwave oven to expose the germ to an averaged field.

Table 3
Estimated operating costs for a dry-grind plant modified to produce 4.1 million kg/year of crude corn oil.

	US$/year
Raw materials	
Dry-milled germ	2,690,000
Cellulase	750,000
Water	19,620
Utilities	
Electricity	328,000
Chilled water	378,252
Labor and supplies	
Labor dependent[b]	396,000
Facility dependent[a]	375,000

[a] Labor cost was based on 1 operator at $50/h for 7920 h/year.
[b] Total capital investment of $2.6 million was estimated from an equipment purchase cost of $873,000 and installation cost of $1.725 million.

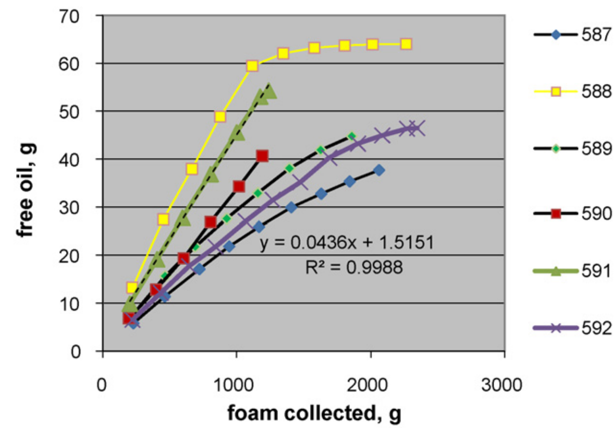

Fig. 2. Total oil extracted by enzymatic extraction from dry-fractionated germ. Run conditions are described in Tables 1 and 2.

inated, and in which no foaming occurred, showed that this step is necessary to release the foam stabilizing compound(s) and form collectable foam. The best free oil yield (run 588) resulted from initially colloid milling the dry-fractionated germ and then drying the dispersion in a vacuum dryer followed by microwave heating, pressure cooking, colloid milling (again) and enzymatic digestion. After 8 min of microwave heating (50% power), the colloid-milled and vacuum-dried germ temperature was 100 °C and after another 8 min of heating (50% power), the final temperature of the germ was 183 °C. The initial milling would be practical for dry-fractionated germ that is initially dry, but difficult to extend with our equipment. The colloid mill cannot grind a suspension with more than 10% solid particles. The subsequent vacuum drying took several days to get a dry substrate for the subsequent extraction steps. An alternative, pressure cooking the un-dried dispersion of colloid-milled germ failed because foaming during the cook caused the contents to vent from the cooking pot. Drying the milled dispersion apparently prevented foaming during the pressure cook in run 588. The yield improvement from colloid milling before heating and cooking may have resulted from better microwave cooking due to a more homogeneous particle mass in the oven. Greater milled particle bulk density and greater surface/volume ratio helped prevent burning that occurred when regular size dry-fractionated germ was heated above 144 °C. The colloid milling and subsequent drying may also have opened channels and voids in the particles which permitted convective heat flow to reduce heat spiking and burning within the milled particles (Tables 1 and 2).

We tried improving microwave cooking effectiveness by adding a second turntable to the oven, located so that the tray holding the dry-milled germ would travel through the widest cycloidal path during a rotational cycle. We hypothesized that this would improve the cooking because the microwave field in the oven was insufficiently uniform to produce the same temperature profile in all of the dry-fractionated germ particles (whose size and composition varied substantially) without burning a significant fraction of the particles—if heated for more than 16 min at 50% power.

The free oil increased linearly with foam collection, as shown for several runs in Fig. 2. The linear fit to a run is also included on the figure. The two runs with the highest oil yields are similar suggesting that the oil fractions of their foams, about 7%, may be near the highest amount that foams produced from dry-fractionated germ dispersions can support.

Heating with microwaves was used because it was the most convenient method to obtain the temperatures required to disrupt the lipid body structure and release the oil from it (Dickey et al., 2007). However, at a commercial scale, overcoming microwave heating's variable deposition of radiant energy might be more expensive than using a convection oven with a fixed temperature. Also, at a larger scale, the foaming problem with pressure cooking finely milled germ could be overcome by venting steam through a valve well above the foam, which is not possible in a commercial cooking pot.

The run in which germ was colloid milled and drained through screens showed that 64% of the protein and 65% of the oil in the colloid-milled germ drained through the screens and thus was contained in particles less than 149 μm in diameter or dissolved. The remaining, larger germ particles, about half the original germ mass, produced 1100 g of foam, a typical amount, after microwave heating to dryness, mixing with water, cooking at 100 °C, further colloid milling and enzyme digestion. Since foam production was not reduced by the soaking and draining the foam stabilizing compound(s) did not dissolve until the germ was heated in a wet medium and digested with the enzyme complex (after milling). The fairly close match of the oil and foam yields suggests that most of the lipid bodies were exposed by germ structure disruption by heating and milling and thus susceptible to the enzymes. The final enzyme digestion step allowed oil and stabilizer release in an aqueous environment where the oil droplets were contained and collected with the foam.

3.4. Flow chart and cost estimation

The measured compositions of the dispersion fractions after cooking, grinding, digestion, and centrifugation were used to prepare a flow sheet for a continuous process, Fig. 3. Process costs were estimated for the aqueous enzymatic extraction using the flow sheet. Because the oil content of dry-fractionated germ is only about half that of wet-mill germ, the non-oil coproduct values, from the non-oil components of the dry-fractionated germ, is important to the total product revenue. The aqueous enzymatic extraction cost estimate takes the dry-fractionated germ as feed at $ 0.11/kg based on a 12.2% protein content and a 14.8% oil content (Johnston et al., 2005).

Table 3 lists estimated operating costs for a dry grind plant modified to produce crude corn oil. For a plant extracting 24 million kg/year of dry-fractionated germ producing 151 million L of ethanol [40 million gallons] per year, the construction cost to add this to a dry-grind plant to extract oil from dry-fractionated germ (not including germ separation), would be $2.6 million. The operating cost would be $1.19/kg of oil. The oil product revenue from the two oil streams would be $2.95 million/year and the total revenue $4.7 million/year based on a (crude) oil selling price of $0.86/kg, a protein price in animal feed of $ 0.40/kg, and the value of the stream to the fermentation of $0.022/kg. The cost of the dry-fractionated germ is 64% of the annual operating cost of $4.2 million/year. The enzyme cost at a

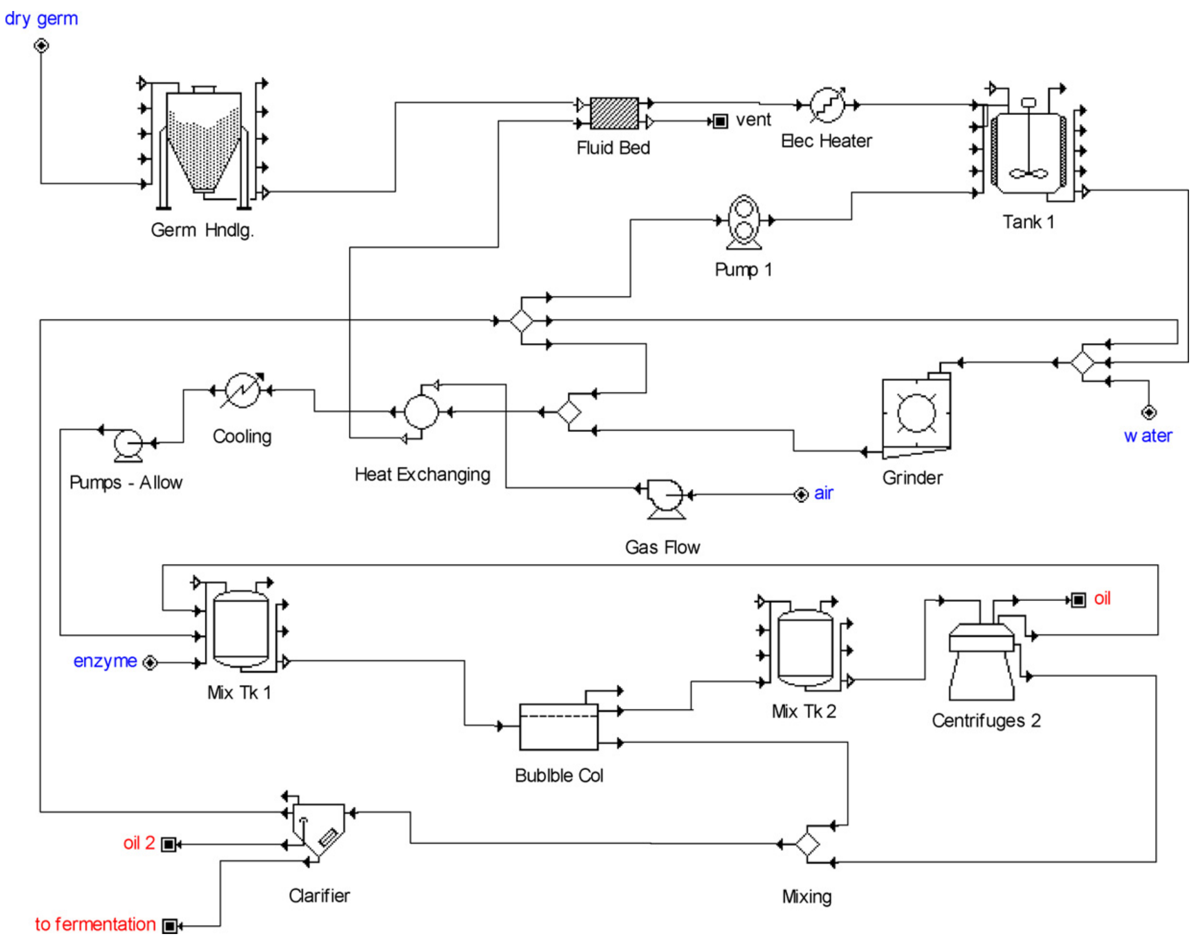

Fig. 3. Aqueous enzymatic extraction of dry-fractionated germ, flow sheet.

dose of 1/80 the dry-fractionated germ mass is estimated to be $750,000 per year, some of the enzyme may be recovered from the digestion step by recycling the non-foam stream from the bubble column and the foam centrate, as shown in the flow sheet, Fig. 3.

4. Conclusions

The extractions described here show that oil can be extracted from dry-fractionated germ using aqueous enzymatic extraction. The germ must be heated to a high enough temperature to weaken the oil bodies and also colloid milled fine enough to allow the enzyme to reduce the structure of the germ so the oil can be released as droplets suspended in the surrounding water. Keeping the oil droplets within the germ until it is suspended in water reduces loss of oil on the equipment internal surfaces. The process cost is compatible with prices for the oil and aqueous enzymatic extraction coproducts.

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